

USSN 07/846,208, filed March 4, 1992; USSN 08/643,810, filed May 6, 1996, now U.S. Pat. No. 6,277,635; and Groux, et al. (1997) Nature 389:737-742; each of which is incorporated herein by reference. Type 1 T-regulatory (Tr1) cells are defined, in part, by their unique cytokine profile: they produce high levels of IL-10, significant levels of TGF- $\beta$  and IFN- $\gamma$ , but no IL-4 or IL-2. Herein, it is investigated whether in vitro differentiation of human Tr1 cells from naive CD4<sup>+</sup> T cells is regulated by cytokines. It is shown that in cord blood T cells, IFN- $\alpha$  induces differentiation of a population of cells with a Tr1-like profile of cytokine production. In contrast, with peripheral blood T cells, both exogenous IL-10 and IFN- $\alpha$  were required for differentiation of Tr1 cells. Cultures with Tr1 cells had a reduced proliferative capacity in response to polyclonal activation, and a suppressed response to alloantigens. Suppression of the alloantigen response was mediated in part by IL-10 and TGF- $\beta$ . The present invention is based, in part, on the definition of conditions for in vitro differentiation of human Tr1 cells. This will facilitate further characterization of this unique T-cell subset and enable their clinical use as cellular therapy to induce tolerance to foreign proteins, e.g., alloantigens.--

Please replace the paragraph on page 14, lines 5-23, with the following rewritten paragraph:

--A number of experiments were designed to determine the effects of IL-10, IFN- $\alpha$ , and IL-15 on the differentiation of IL-10-producing T cells. Efforts have focussed on aspects of the differentiation system described by Sornasse, et al. (1996) J. Exp. Med. 184:473-483) which involves co-culture of CD4<sup>+</sup> T cells with irradiated L-cells, expressing CD32, CD58, and CD80, in the presence of anti-CD3, IL-2, and/or IL-15, and polarizing cytokines. Following two rounds of stimulation, cells are collected, stimulated with  $\alpha$ CD3 and  $\alpha$ CD28, and analyzed by intra-cytoplasmic staining and ELISA for the production of IL-10, IL-4, IL-2, and IFN- $\gamma$ . Experiments were initiated with CD4<sup>+</sup> T cells derived from cord blood, which cells have an innate ability to produce high levels of IL-10. Addition of IFN- $\alpha$  resulted in a significant, e.g., 5-6 fold, increase in the percentage of IL-10-positive cells compared